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13. ABSTRACT (Maximum 200 Words)

Our objective is to develop a rodent model for human DCIS and LCIS in which lesions of diverse phenotypes can be induced and characterized and their malignant potential studied in a linear manner. We tested the effect of the infusion of EGF, Heregulin, FGF, or IGF1 on the proliferation of mammary glands in ovariectomized rats. Infusion of EGF, Her, FGF, IGF1 resulted in end bud formation and lobuloalveolar development within 72 hours. Intraductal proliferation resulted only in rats treated with IGF1. We determined that treatment with growth factors alone would support neoplastic transformation in ovariectomized rats. EGF or Her were infused into the mammary glands of ovariectomized rats and treated with the chemical carcinogen N-methyl-N-nitrosourea and ovaries transplanted. 75% of the rats in both groups developed mammary cancers. These results indicate that infusion of growth factors into the mammary induces proliferation of different kinds of structures, including end buds, lobuloalveolar structures and intraductal proliferation's and that the growth factors in ovariectomized rats can support neoplastic mammary transformation. Our future plans are to select lesions induced in the presence of different growth factors, devlop transplantation lines and characterize their biological behavior and their genetic changes.

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Table of Contents

Introduction

Our goals are to develop methods for the induction of a large number of ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS) lesions with different phenotypes, be able to detect the lesions in situ and characterize the lesions as to their cancerous potential, hormone dependence or independence, and genetic changes. DCIS and LCIS are intraductal and intralobular hyperplasias. Proliferation of these cells is a prerequisite to carcinoma. However, intraductal proliferation with the exception of the terminal end bud occurring in peripubertal rats is extremely rare and has not been found in the terminal ductal structures during development or under experimental conditions. Our laboratory has made the novel finding that treatment of rats by infusing the mammary ducts with a combination of epidermal growth factor, cortisol, and cholera toxin causes extensive intraductal proliferation in the terminal ducts with a proliferation labeling index as high as 75% (1,2). Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family (FGF 7) that is secreted by stromal cells and acts on epithelial cells. Treatment of intact rats with KGF causes massive intraductal hyperplasias (3). KGF can cause ductal growth and intraductal hyperplasias in ovariectomized mice. Concomitant treatment with estradiol and progesterone plus KGF increases intraductal hyperplasias(). The intraductal hyperplasias regress after withdrawal of the mitogenic stimulus. We think that it should be possible to induce intraductal/intraalveolar hyperplasias by a variety of means and then treat with different chemical carcinogens to cause a large number of immortalized transformed phenotypes resembling DCIS and LCIS (4,5). These unique mitogens that cause intraductal proliferation have not been used, to our knowledge, in combination with ductal or alveolar mitogens in attempts to develop DCIS and LCIS. We believe that these treatments should result in an expanded pool of target cells for DCIS and LCIS which can then be transformed to preneoplastic and neoplastic states with well known mammary carcinogens such as N-methyl-N-nitrosourea (4), N-ethyl-nitrosourea, dimethylbenz(a)anthracene, or radiation.

Body

Task 1. Development of methods for the induction of DCIS and LCIS in inbred Lewis rats (months 1-12).

a) Determine which hormones or growth factors or combinations induce intraductal or intralobubular hyperplasia (3 rats/treatment).
b) Quantitate proliferation by immunocytochemistry and confirm intraductal or intralobular hyperplasia by histology.

We have found that direct infusion of growth factors into the mammary ducts results in intraductal and intralobular hyperplasias. Methods for the induction of intraductal and intralobular proliferation have been developed in rats using the mammogenic hormones, estradiol (1o ng), progesterone (5υg), prolactin ((5ug), and growth hormone (5 υg) administered singly or in combination. The growth

factors, epidermal growth factor (EGF, 100ng, 1 υg, 5 υg), Heregulin (Her, 100 ng, 1υg) keratinocyte growth factor (KGF, 100ng, 400ng), fibroblast growth factor α (FGF, 1 υg, 5 υg), insulin like growth factor 1 (IGF1, 1υg, 5υg) and cholera toxin (CT, 1ng, 10ng, 100 ng) are agents that have been shown to increase proliferation within ducts or lobules. Intact female Lewis rats were divided into groups of 3 rats per group at 5 weeks of age and mammogenic hormones or growth factors alone or in combination with CT were infused (15 υl) through the nipples. Groups of rats were sacrificed at 24 and 72 hours. Mammary whole mounts were prepared and examined. The results indicated that lobular and ductal proliferation was induced following the infusion of mammogenic hormones and growth factors. The infusion of growth factors resulted in intraductal proliferation that was not induced by mammogenic hormones. The morphology of the ducts, lobules, and intraductal proliferations was confirmed by histological examination, proliferation was confirmed by immunocytochemistr for bromodeoxyuridine (BRDU) uptake. We tested the effect of the infusion of EGF (1 υg, 5υg), FGF (1 υg, 5 υg) or IGF1 on the proliferation of mammary glands in ovariectomized rats. Five-week-old rats were ovariectomized. Two weeks later the growth factors were infused into the mammary glands of 3 rats per group. Rats were sacrificed at 24 and 72 hours after treatment. Two hours before termination the rats were injected with BRDU. EGF, FGF, or IGF1 resulted in end bud formation and lobuloalveolar development within 72 hours after infusion. Intraductal proliferation resulted only in rats treated with IGF1. carcinogenesis.

c) Transform treatment induced hyperplasia by administration of MNU (5 rats/group)

We determined whether treatment with growth factors alone would support neoplastic transformation in ovariectomized rats. mammary carcinogenesis. Seven rats were used as intact controls. Female Lewis rats were ovariectomized at 6 weeks of age. Three weeks after ovariectomy the rats were divided into 3 groups and the mammary glands were infused with phosphate buffered saline, 1 υg of EGF, or 1 υg of Her. Twenty-four hours after infusion the ovariectomized rats were treated with 50 mg/kg body weight of the direct acting carcinogen N-methyl-N-nitrosourea and 1/2 of an ovary transplanted subcutaneously to promote mammary carcinogenesis. Six months after treatment with carcinogen, 7 of 7 intact control rats developed mammary cancer, 2 of 6 ovariectomized rats treated with phosphate buffered saline developed mammary cancer, 8 of 10 EGF treated and 8 of 10 Her treated rats developed mammary cancer. Mammary cancers were confirmed by histopathology. Immunocytochemical analysis for the presence of estrogen receptor and progesterone receptor indicated that all the mammary cancers were hormone dependent. These result indicate that the growth factors EGF and Her are capable of supporting mammary neoplastic transformation in ovariectomized rats.

Key Research Accomplishments

- Demonstrated that infusion of mammogenic hormones infused through the nipple of the mammary gland results in rapid proliferation of the mammary epithelium within 72 hours.
- Demonstrated that infusion of EGF, FGF. Her, or IGF-1 into ovariectomized rats results in the rapid proliferation of ducts and lobules.
- Infusion of IGF1 into ovariectomized rats results in the induction of intraductal proliferations.
- Demonstrated that the growth factors EGF and Her are capable of supporting mammary neoplastic transformation in ovariectomized rats.

Reportable Outcomes

None

Conclusions

Taken together, these results indicate that proliferation of the mammary epithelium can be induced quickly by infusion of mammogenic hormones and growth factors. The pattern of proliferation varies with the hormone or growth factor used. The induced proliferations can be neoplastically transformed with a chemical carcinogen. These findings suggest that it should be feasible to induce proliferation of the mammary epithelium with different agents and neoplastically transform them resulting in lesions with different morphologies and different phenotypes and genotypes. In the next grant period, it is our plan to induce proliferation of the mammary epithelium with different mammogenic hormones and growth factors, identify transformed lesions in situ, and transplant them to syngeneic hosts and develop stable lines with different morphologies and neoplastic potentials.

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